A flow chart for array-based detection of gene expression
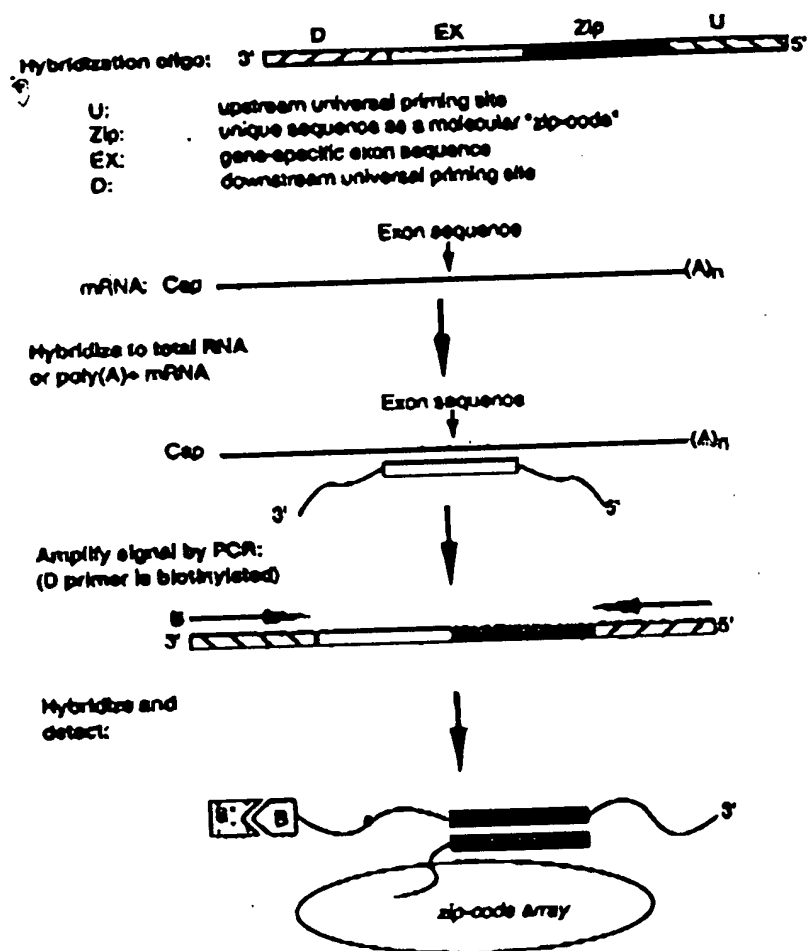

Hybridization oligo: 3' [D] [EX] [Zip] [U] 5'

U: upstream universal priming site
Zip: unique sequence as a molecular "zip-code"
EX: gene-specific exon sequence
D: downstream universal priming site

Exon sequence

mRNA: Cap ———————————————(A)n

Hybridize to total RNA
or poly(A)+ mRNA

Exon sequence

Cap ———————————————(A)n

3'   5'

Amplify signal by PCR:
(D primer is biotinylated)

5'
3' [   ] 5'

Hybridize and
detect:

B:B

zip-code array

Figure 1

A flow chart for array-based detection of RNA alternative splicing

Hybridization oligo: 3' [D SJ Zip U] 5'

U: upstream universal priming site
Zip: unique sequence as a molecular "zip-code"
SJ: gene-specific splice junction
D: downstream universal priming site

Splice junction

mRNA: Cap ————————————————— (A)n

Hybridize to total RNA
or poly(A)+ mRNA

Splice junction

Cap ————————————————— (A)n

3'   3'

Amplify signal by PCR:
(D primer is biotinylated)

3' [   ] 5'

Hybridize and
detect:

zip-code array
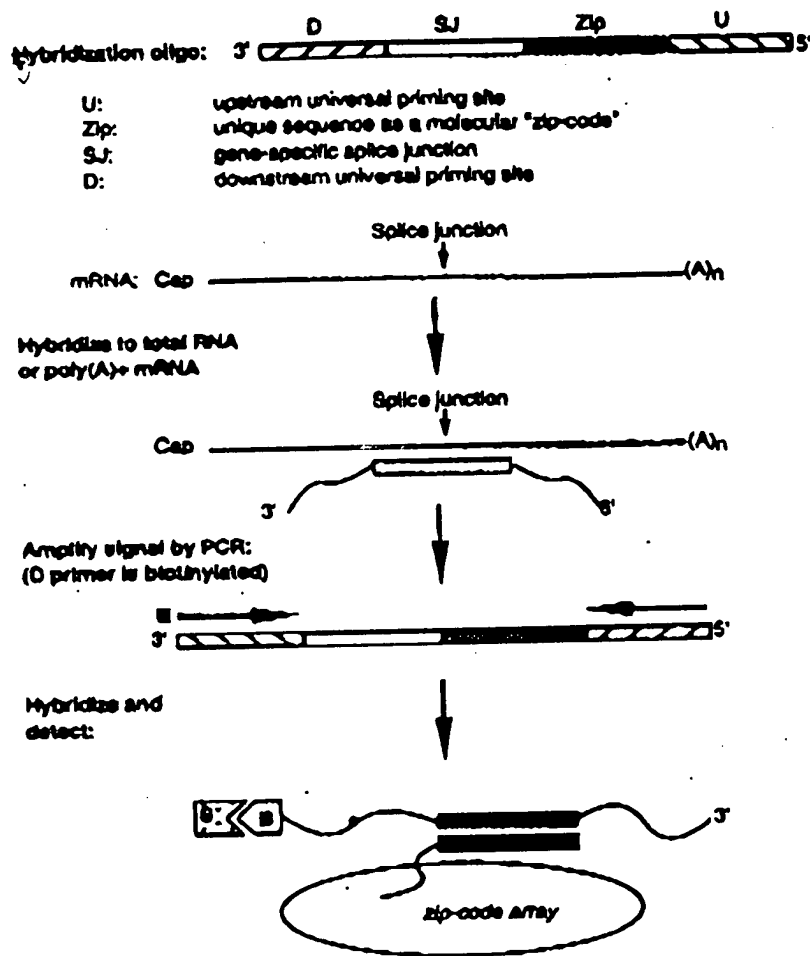
Figure 2

Genome-wide gene expression profiling using oligo-ligation strategy
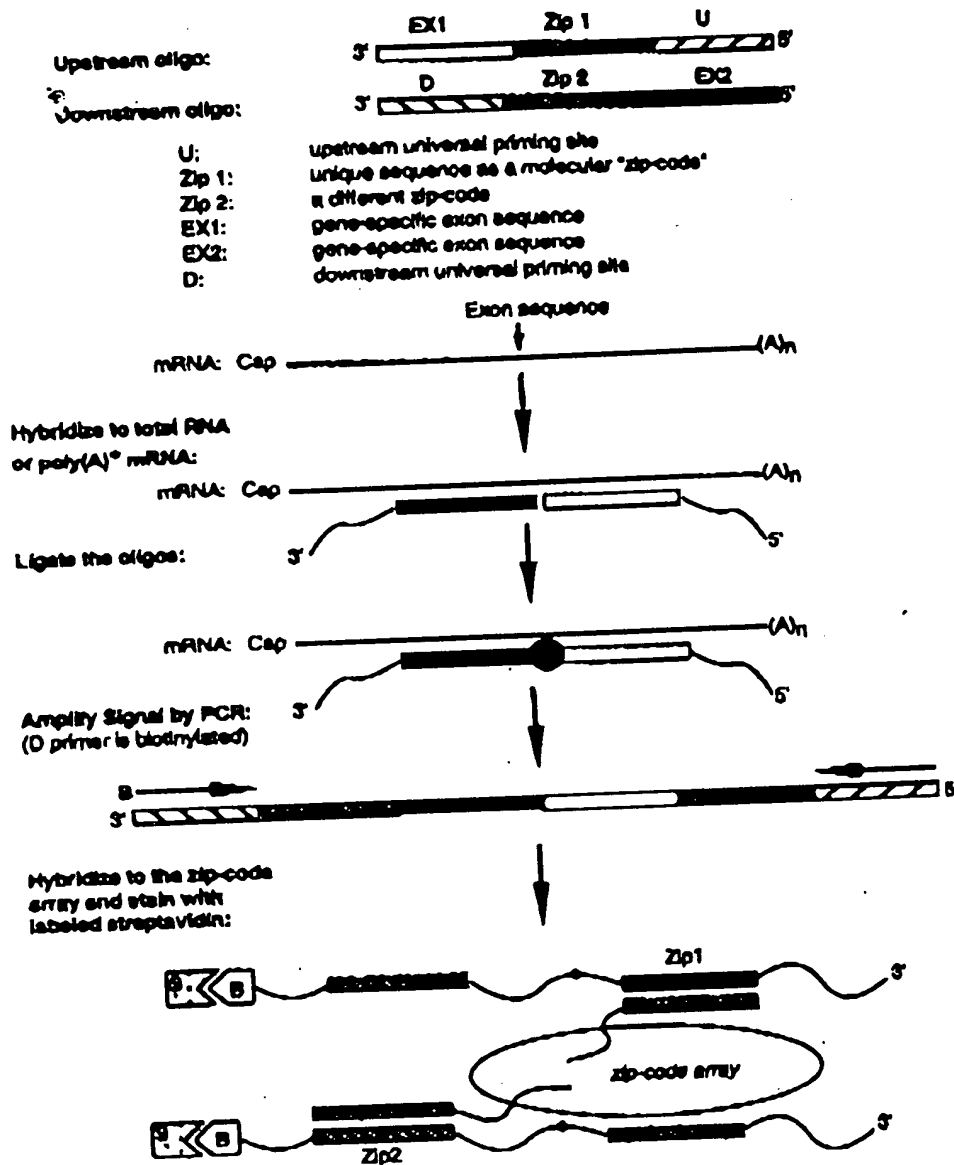
Figure 3

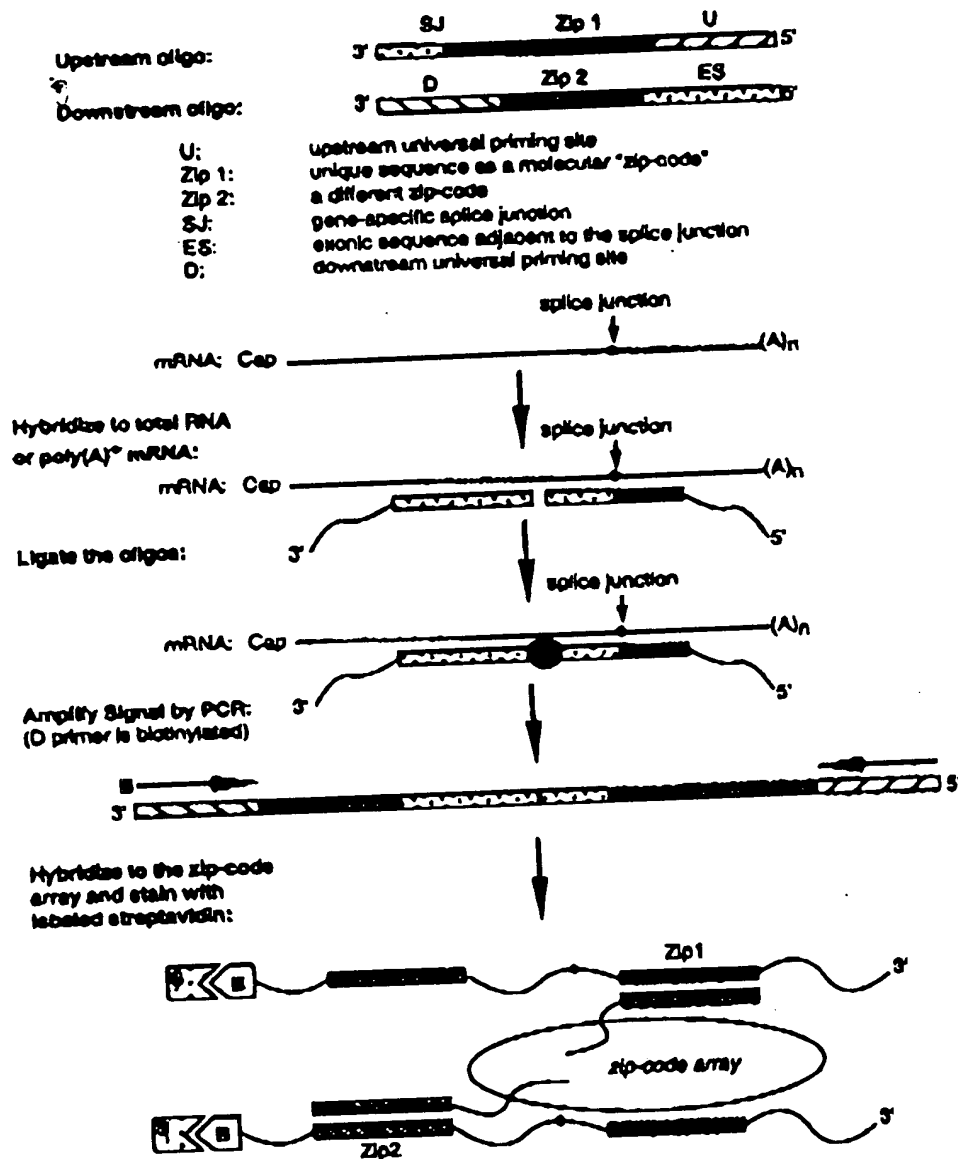**Genome-wide RNA alternative splicing monitoring using oligo-ligation strategy**

Upstream oligo:

| SJ | Zip 1 | U |

Downstream oligo:

| D | Zip 2 | ES |

U: upstream universal priming site
Zip 1: unique sequence as a molecular "zip-code"
Zip 2: a different zip-code
SJ: gene-specific splice junction
ES: exonic sequence adjacent to the splice junction
D: downstream universal priming site

splice junction

mRNA: Cap ————————————— (A)n

Hybridize to total RNA
or poly(A)+ mRNA:

splice junction

mRNA: Cap ————————————— (A)n

Ligate the oligos:

splice junction

mRNA: Cap ————————————— (A)n

Amplify Signal by PCR:
(D primer is biotinylated)

Hybridize to the zip-code
array and stain with
labeled streptavidin:

Zip1

zip-code array

Zip2

Figure 4

Figure 5

Target_U   Zip 1   U

Upstream oligo: 3' [_____] 5'

D   Zip 2   Target_D

Downstream oligo: 3' [_____]

Target_M

Middle oligo: 5' [____] 5'

U: upstream universal priming site
Zip 1:   unique sequence as a molecular "zip-code"
Zip 2:   a different zip-code
Target_U: upstream target-specific sequence
Target_D: downstream target-specific sequence
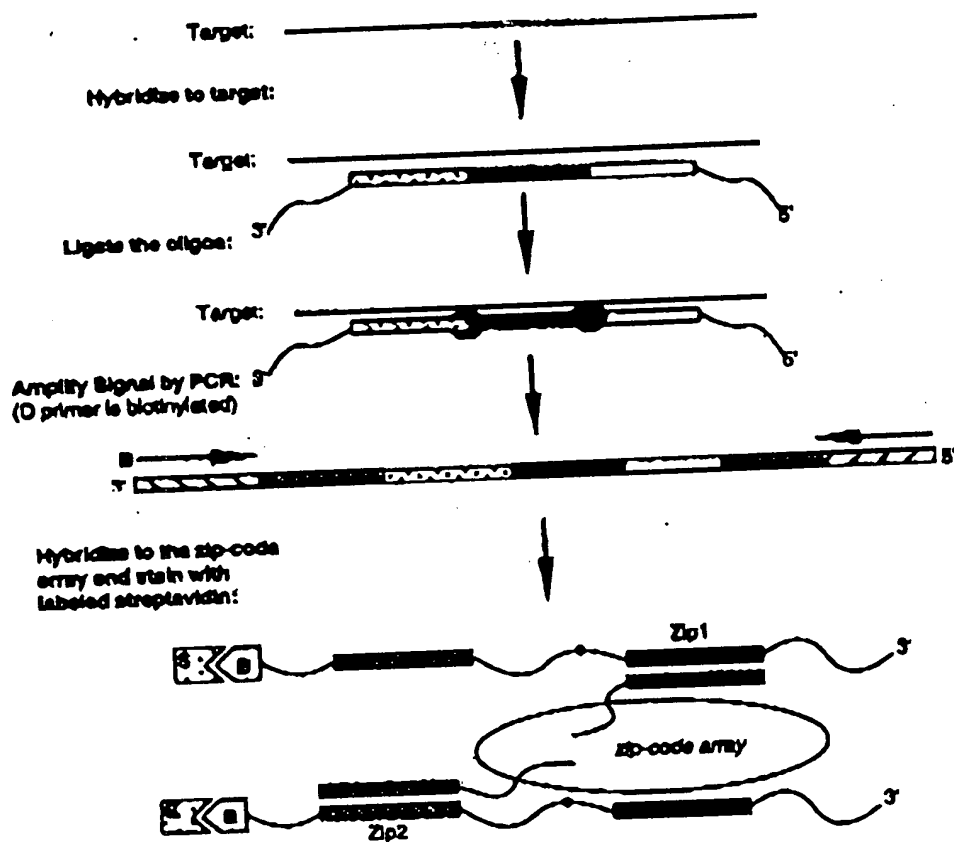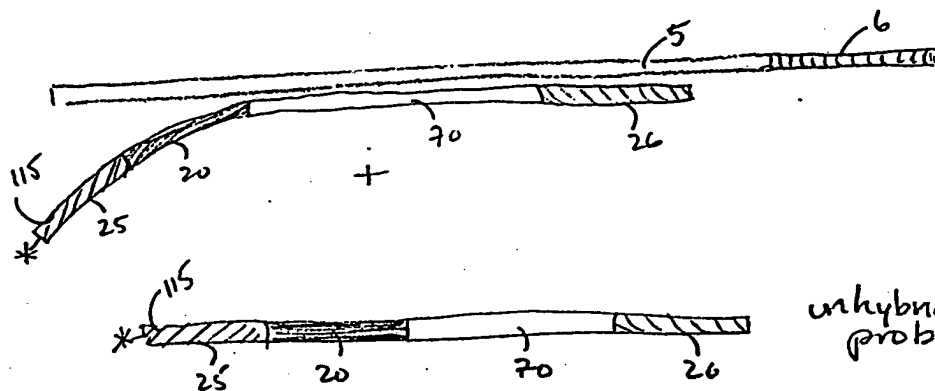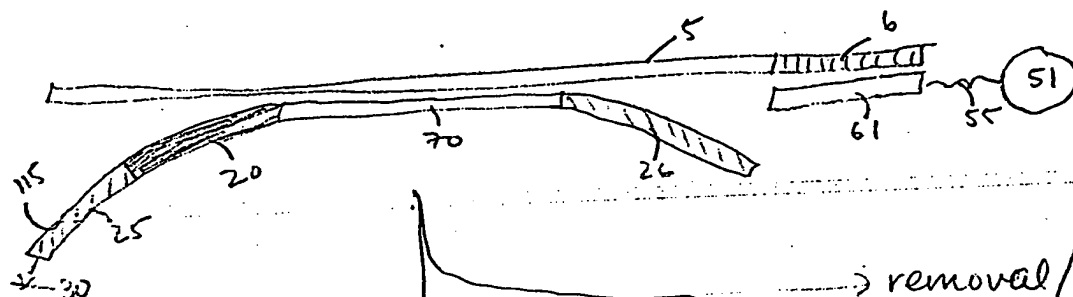Target_M: middle target-specific sequence
D: downstream universal priming site

Target: _____

Hybridize to target:

Target: _____

Ligate the oligos: 3'

Target: _____

Amplify Signal by PCR: 3'
(D primer is biotinylated)

B _____ 5'
3' _____

Hybridize to the zip-code
array and stain with
labeled streptavidin:

Zip1

zip-code array

Zip2

Figure 6

unhybridize
probes

removal/wash
of unhybridized
probes

denature

amplify

Fig 7

5

100

30

25

20

15

16

110

26

35

ligation

denaturation

addition
of exonuclease

30 115 25 20 70 26 35

+

110 16 26 35
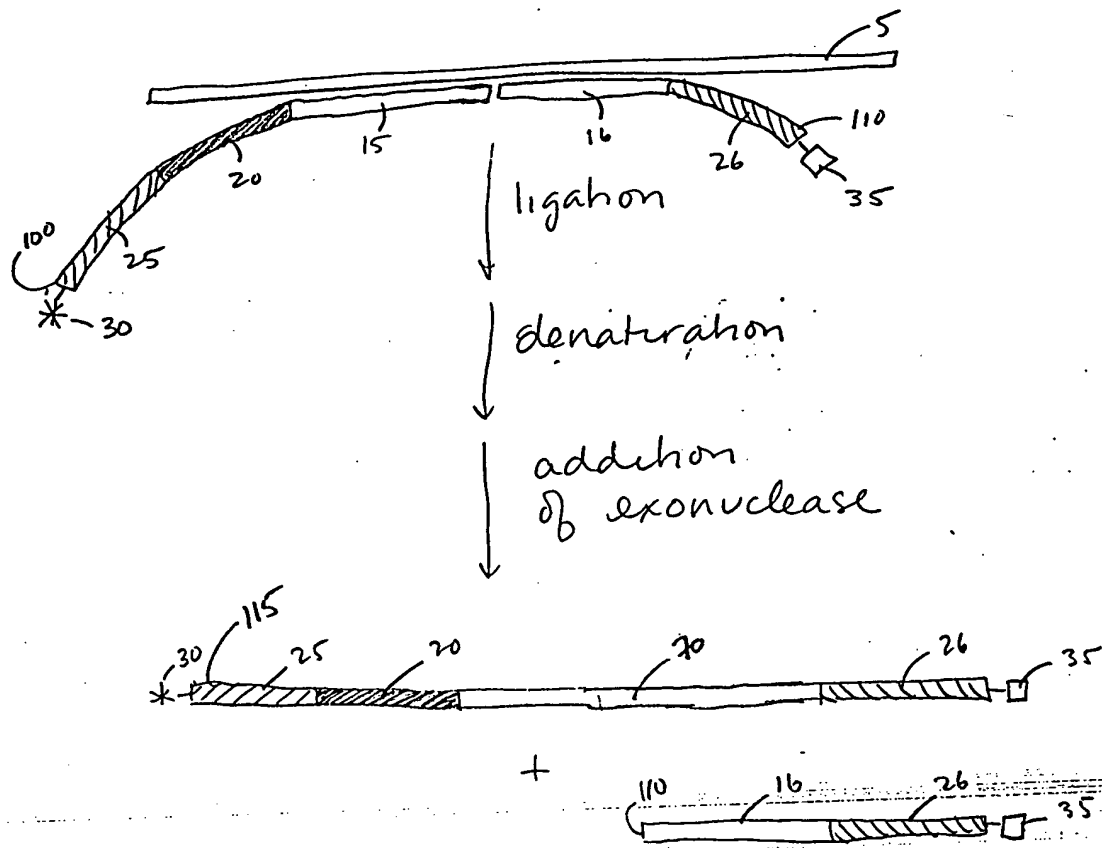
addition to
array,
wash away unbound

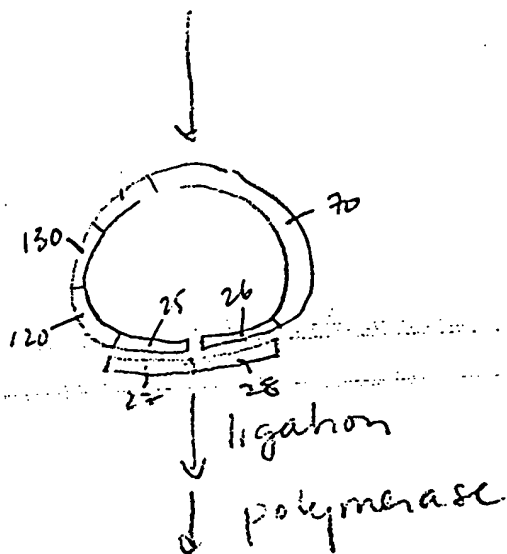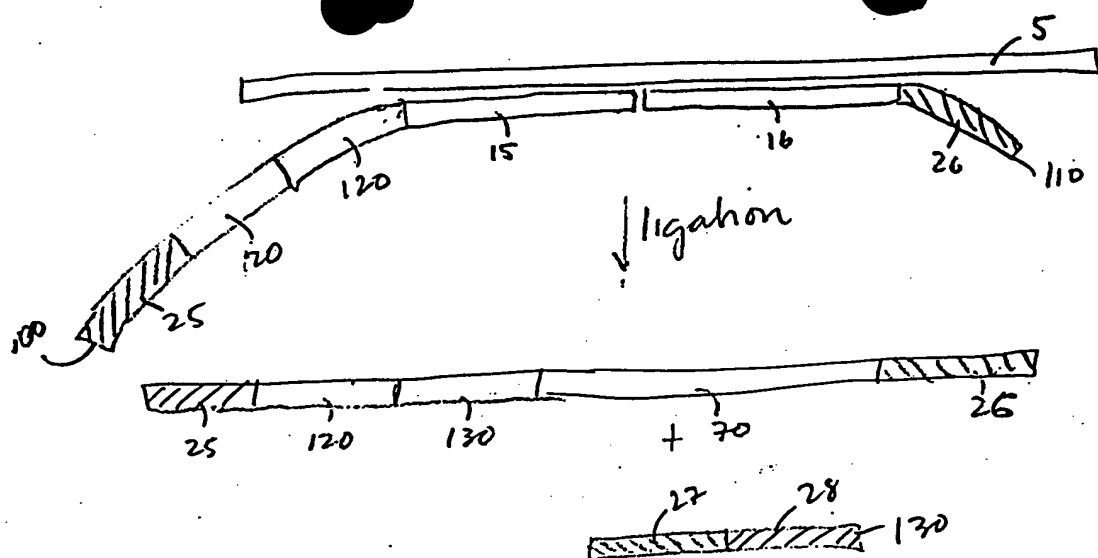40

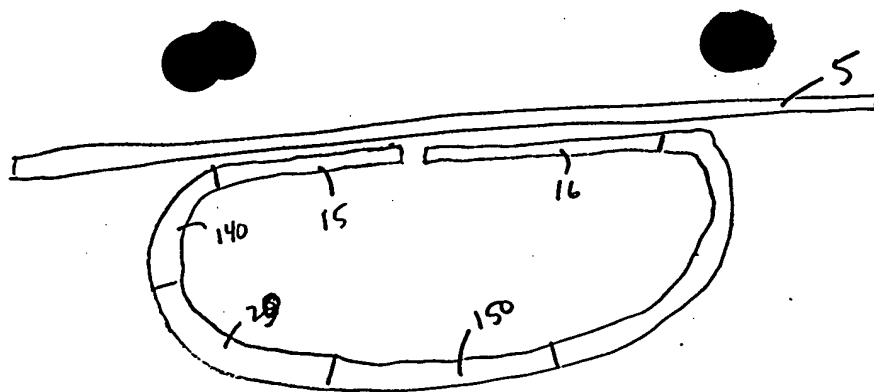50 55 60

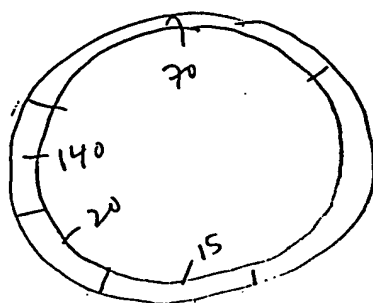25 20 70 26 35

30

FIG 8

ligation

ligation

polymerase
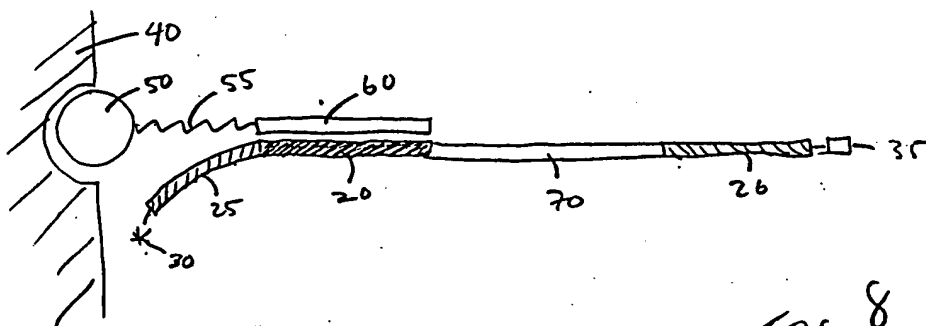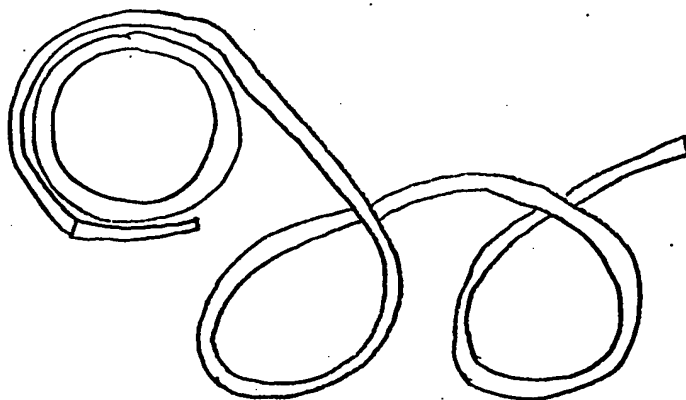
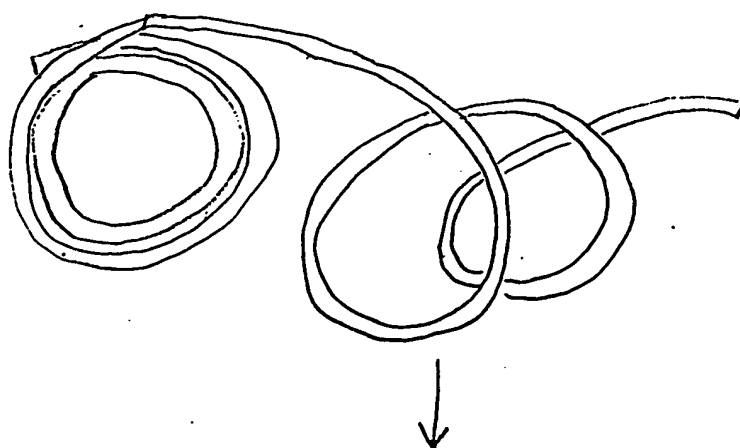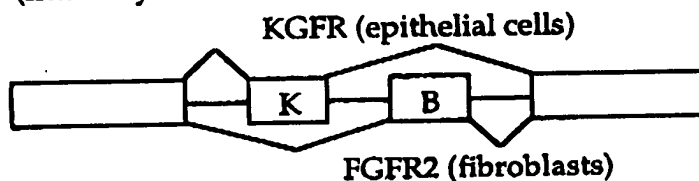Fig 9

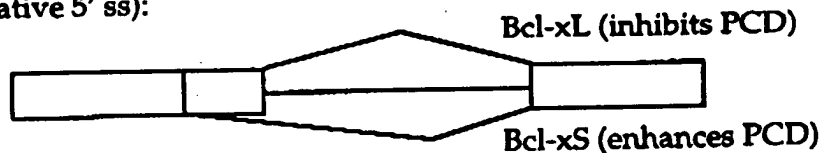ligation, denaturation

addition of primer, extension

FIG 10

Alternative Splicing Targets Selected for Microarray Analysis

1. GAPDH (constitutive splicing control, signal normalization).

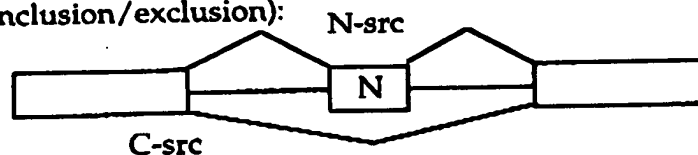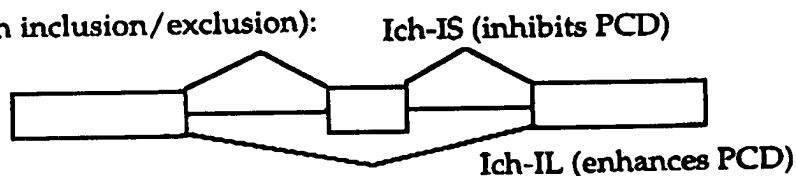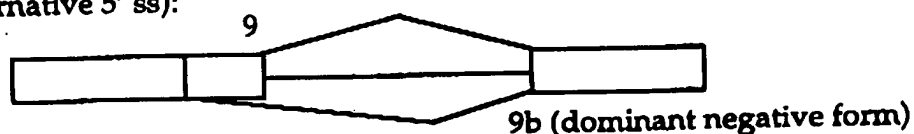2. FGFR2/KGF (mutually exclusive exons, internal cell type control):

KGFR (epithelial cells)

K   B

FGFR2 (fibroblasts)

3. Bcl-x (alternative 5' ss):

Bcl-xL (inhibits PCD)

Bcl-xS (enhances PCD)

4. c-src (exon inclusion/exclusion):

N-src

N

C-src

5. CASP2 (exon inclusion/exclusion):

Ich-IS (inhibits PCD)

Ich-IL (enhances PCD)

6. CASP9 (alternative 5' ss):

9

9b (dominant negative form)

7. Fyn (src family tyrosine kinase, mutually exclusive exons):

brain-specific

7A   7B

thymus specific
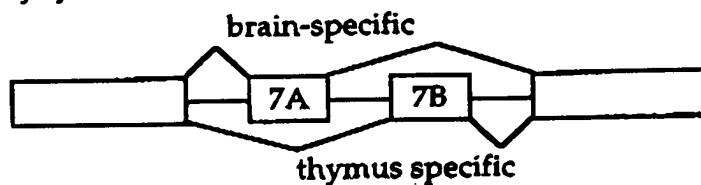
8. NOS1 (alternative promoters/alternative 5'ss):

FIG
11